What is claimed is:

1. A method of preparing achiral NF-κB specific aptamers comprising the steps of:

(a) synthesizing a random phosphodiester oligonucleotide combinatorial library wherein constituent oligonucleotides comprise at least a set of 5' and 3' PCR primer nucleotide sequences flanking a randomized nucleotide sequence;

(b) amplifying the library enzymatically using a mix of four nucleotides, wherein at least a portion of the total quantity of at least one but no more than three of the nucleotides is modified, to form a modified oligonucleotide combinatorial library;

(c) contacting the modified oligonucleotide combinatorial library with a NF-kB constituent protein and isolating a subset of oligonucleotides binding to the protein;

(e) amplifying the subset of binding oligonucleotides enzymatically using a mix of four nucleotide substrates, wherein at least a portion of the total quantity of at least one but no more than three of the nucleotides is modified, to form a modified oligonucleotide sub-library; and

(f) repeating steps (c) - (e) iteratively until at least one aptamer comprising a modified oligonucleotide population of defined sequence is obtained.

2. The method of claim 1, wherein the modified nucleotide comprises a phosphorothioate or phosphorodithioate.

3. The method of claim 2, wherein the modified nucleotide is member of the group: dATP(αS), dTTP(αS), dCTP(αS), dGTP(αS),dATP(S_2), dTTP(S_2), dCTP(S_2) and dGTP(S_2).

4. The method of claim 1, wherein the modified nucleotide is dA.

5. An aptamer specific for NF-kB or a portion therof essentially homologous to the sequences of oligonucleotides identified by SEQ ID NOS.: 20 - 28 wherein one or more nucleotides have at least one thiophosphate or dithiophosphate group.

6. The aptamer of claim 5, wherein a portion of all dA or dT nucleotides are thiophosphates or dithiophosphates.

7. The aptamer of claim 6, wherein the substantially all dA nucleotides are thiophosphates or dithiophosphates.

8. An aptamer specific for NF-κB or constituents thereof comprising a nucleotide sequence essentially homologous to a nucleotide sequences of the formula:

GGGCG T ATAT G* TGTG GCGGG GG (SEQ ID NO.: 28)

wherein at least one nucleotide is an achiral thiophosphate or a dithiophosphate.

9. The aptamer claim 8, wherein a portion of the phoshate sites are replaced with phosphorothioate groups.

10. The aptamer of claim 8, wherein no more than three adjacent phosphate sites are replaced with phosphorothioate groups.

11. A method of post-selection aptamer modification comprising the steps of:

(a) identifying a first generation target binding aptamer comprising a known sequence of nucleotide bases;

(b) substituting modified achiral nucleotides for one or more selected nucleotides in the sequence, wherein the substitution results in modified second generation

aptamers of the same base sequence as the first generation but with increased nuclease resistance; and

(c) determining the modifications resulting in a relative second generation binding efficiency to the target equal to or greater than the first generation.

12. The method of claim 11, wherein the modified achiral nucleotide is an achiral thiomonophosphate

13. The method of claim 11, wherein the modified achiral nucleotide is a dithiophosphate.

14. The method of claim 11, wherein the target binding aptamer is specific for NF-kB or constituents thereof.

15. An aptamer specific for NF-kB or constituents thereof essentially homologous to the sequences of oligonucleotides in the group consisting of:

a) single stranded oligonucleotides identified by SEQ ID NO.: 17, wherein between one and six of the nucleotides are dithiophosphates;

b) double stranded oligonucleotides in accordance with the sequence identified by SEQ ID NO.: 17, wherein between one and ten of the nucleotides are dithiophosphates;

c) single stranded oligonucleotides identified by SEQ ID NO.: 29, wherein between one and six of the nucleotides are dithiophosphates; and

d) double stranded oligonucleotides in accordance with the sequence identified by SEQ ID NO.: 29, wherein between one and ten of the nucleotides are dithiophosphates.

16. The aptamer of claim 15, essentially homologous to and having the thioate substitutions of the oligonucleotides identified by SEQ ID NO.: 30 - 39.

17. The aptamer of claim 15, essentially homologous to an oligonucleotide sequence of the formula:

5'-CCAGGAGAT$_{S2}$T$_{S2}$CCAC-3'

3'-GG$_{S2}$TCC$_{S2}$TC$_{S2}$TAAGG$_{S2}$TG-5' (SEQ ID NO.: 39).

18. An achiral oligonucleotide that specifically binds a target comprising a sequence of nucleotides one or more nucleotides in sequence are thiophosphate modified.

19. The achiral oligonucleotide of claim 18, wherein the one or more thio-modified nucleotide in the achiral oligonucleotide is selected from the group consisting of dATP(S), dTTP(S), dGTP(S) and dCTP(S).

20. The achiral oligonucleotide of claim 18, wherein not all like nucleotides have a thiomodified phosphate group.

21. The achiral oligonucleotide of claim 18, wherein only one nucleotide has a thiomodified phosphate group.

22. The achiral oligonucleotide of claim 18, wherein two nucleotides have a thiomodified phosphate group.

23. An achiral oligonucleotide that specifically binds a target comprising a sequence of nucleotides wherein one or more of the nucleotides in sequence are dithiophosphates.

24. The achiral oligonucleotide of claim 23, wherein the one or more thio-modified nucleotide in the achiral oligonucleotide is selected from the group consisting of dATP(S), dTTP(S), dGTP(S) and dCTP(S).

25. The achiral oligonucleotide of claim 23, wherein not all like nucleotides have a thiomodified phosphate group.

26. The achiral oligonucleotide of claim 23, wherein only one nucleotide has a thiomodified phosphate group.

27. The achiral oligonucleotide of claim 23, wherein two nucleotides have a thiomodified phosphate group.

28. The achiral oligonucleotide of claim 23, wherein between approximately 10 and 80% of the nucleotides in the sequence are thiomodified.

29. A process for fractionating oligonucleotides with varying degrees of thiolation comprising the steps of:

a) dissolving a crude thiolated oligonucleotide mixture in a starting solvent;

b) loading the thiolated oligonucleotide containing solvent onto an anion-exchange column; and

c) eluting the thiolated oligonucleotide with a buffered solution comprising a salt gradient.

30. The process of claim 29, wherein the anion-exchange column is a Mono Q column.

31. The process of claim 29, wherein the salt gradient is from approximately 0 to 100% NaCl in a buffer with an approximate pH of around 8.0.

32. An aptamer prepared by the method of claim 1.

33. An aptamer prepared by the method of claim 11.

34. A pharmaceutical preparation comprising an aptamer prepared in accordance with the method of claim 1.

35. A pharmaceutical preparation comprising an aptamer prepared in accordance with the method of claim 11.

36. A biological chip plate comprising a contiguous substrate to which a selectively partially thiolated aptamer is attached.

37. The biological chip of claim 23, in which the attachment of the selectively thiolated aptamer is by photolithography.

38. The biological chip of claim 23, wherein the selectively thiolated aptamer is NF-kB specific.

39. A method of assaying protein:protein or protein:protein:DNA interactions using the biological chip of claim 25.

aptamers of the same base sequence as the first generation but with increased nuclease resistance; and (c) determining the modifications resulting in a relative second generation binding efficiency to the target equal to or greater than the first generation.

12. The method of claim 11, wherein the modified achiral nucleotide is an achiral thiomonophosphate

13. The method of claim 11, wherein the modified achiral nucleotide is a dithiophosphate.

14. The method of claim 11, wherein the target binding aptamer is specific for NF-kB or constituents thereof.

15. An aptamer specific for NF-kB or constituents thereof essentially homologous to the sequences of oligonucleotides in the group consisting of:

a) single stranded oligonucleotides identified by SEQ ID NO.: 17, wherein between one and six of the nucleotides are dithiophosphates;

b) double stranded oligonucleotides in accordance with the sequence identified by SEQ ID NO.: 17, wherein between one and ten of the nucleotides are dithiophosphates;

c) single stranded oligonucleotides identified by SEQ ID NO.: 29, wherein between one and six of the nucleotides are dithiophosphates; and

d) double stranded oligonucleotides in accordance with the sequence identified by SEQ ID NO.: 29, wherein between one and ten of the nucleotides are dithiophosphates.

16. The aptamer of claim 15, essentially homologous to and having the thioate substitutions of the oligonucleotides identified by SEQ ID NO.: 30 - 39.

17. The aptamer of claim 15, essentially homologous to an oligonucleotide sequence of the formula:

5'-CCAGGAGAT$_{S2}$T$_{S2}$CCAC-3'

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18. An achiral oligonucleotide that specifically binds a target comprising a sequence of nucleotides one or more nucleotides in sequence are thiophosphate modified.

19. The achiral oligonucleotide of claim 18, wherein the one or more thio-modified nucleotide in the achiral oligonucleotide is selected from the group consisting of dATP(S), dTTP(S), dGTP(S) and dCTP(S).

20. The achiral oligonucleotide of claim 18, wherein not all like nucleotides have a thiomodified phosphate group.

21. The achiral oligonucleotide of claim 18, wherein only one nucleotide has a thiomodified phosphate group.

22. The achiral oligonucleotide of claim 18, wherein two nucleotides have a thiomodified phosphate group.

23. An achiral oligonucleotide that specifically binds a target comprising a sequence of nucleotides wherein one or more of the nucleotides in sequence are dithiophosphates.

24. The achiral oligonucleotide of claim 23, wherein the one or more thio-modified nucleotide in the achiral oligonucleotide is selected from the group consisting of dATP(S), dTTP(S), dGTP(S) and dCTP(S).

25. The achiral oligonucleotide of claim 23, wherein not all like nucleotides have a thiomodified phosphate group.

26. The achiral oligonucleotide of claim 23, wherein only one nucleotide has a thiomodified phosphate group.

27. The achiral oligonucleotide of claim 23, wherein two nucleotides have a thiomodified phosphate group.

28. The achiral oligonucleotide of claim 23, wherein between approximately 10 and 80% of the nucleotides in the sequence are thiomodified.

29. A process for fractionating oligonucleotides with varying degrees of thiolation comprising the steps of:

a) dissolving a crude thiolated oligonucleotide mixture in a starting solvent;

b) loading the thiolated oligonucleotide containing solvent onto an anion-exchange column; and

c) eluting the thiolated oligonucleotide with a buffered solution comprising a salt gradient.

30. The process of claim 29, wherein the anion-exchange column is a Mono Q column.

31. The process of claim 29, wherein the salt gradient is from approximately 0 to 100% NaCl in a buffer with an approximate pH of around 8.0.

32. An aptamer prepared by the method of claim 1.

33. An aptamer prepared by the method of claim 11.

34. A pharmaceutical preparation comprising an aptamer prepared in accordance with the method of claim 1.

35. A pharmaceutical preparation comprising an aptamer prepared in accordance with the method of claim 11.

36. A biological chip plate comprising a contiguous substrate to which a selectively partially thiolated aptamer is attached.

37. The biological chip of claim 23, in which the attachment of the selectively thiolated aptamer is by photolithography.

38. The biological chip of claim 23, wherein the selectively thiolated aptamer is NF-kB specific.

39. A method of assaying protein:protein or protein:protein:DNA interactions using the biological chip of claim 25.

THIO-MODIFIED APTAMER SYNTHETIC METHODS AND COMPOSITIONS

ABSTRACT

The present invention provides a method for concurrent achiral nucleotide modification and amplification using PCR. Provided by this method are NF-kB specific thioaptamers of novel sequence. This invention further provides methods of post-selection aptamer modification wherein one or more selected nucleotides of aptamers of known sequence are substituted with modified achiral nucleotides, particularly achiral thiophosphate nucleotides, wherein the substitution results in increased nuclease resistance while retaining binding efficiency and selectivity. Thiosubstitution of post-selection aptamers with specificity for the nuclear factor, NF-κB, produced in accordance with this method have increased binding affinity and specificity in addition to nuclease resistance. Also provided are methods for fractionating oligonucleotides depending on their degree of thiosubstitution by anion exchange chromatography.